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LIGNIFICATION OF XYLEM FIBRES IN PARKINSONIA ACULEATA

Flora Murray Scott

Parkinsonia aculeata,[1] one of the palo verdes of the southwestern desert, is commonly cultivated in Los Angeles County, and in the Palm Springs region. It is a handsome tree, freely branching, with a wealth of yellow flowers in early summer (5). The formation of cork is confined almost entirely to the main trunk, which, in consequence, alone lacks the gay green color to which the palo verde owes its name.

Microscopic examination of the switch-like stem reveals such obvious xerophytic characters as the heavy cutinization of the persistent epidermis, the sunken stomata, the presence of a hypodermal layer of water-containing cells, and the development of a photosynthetic cortex. A striking feature of the stem is the abundance of starch throughout the completely lignified xylem.

The present paper is concerned with the development of the starch-containing fibres which make up the bulk of the xylem tissue.

Observations

In the xylem of *Parkinsonia,* seasonal rings are well marked, and the wood is made up of the following elements: tracheal tubes, fibres, and medullary rays. No unlignified, thin-walled xylem parenchyma was noted in the differentiated stems. The fibres are of the type termed "substitute fibres" by Eames and MacDaniels (4), i.e., living fibre elements which function in food storage. In *Parkinsonia* occur two types of substitute fibres which

[1] Correction: in a previous paper by the author, "The anatomy of *Cercidium Torreyanum* and *Parkinsonia microphylla*" (Madroño 3: 33–41. 1935), for *Parkinsonia microphylla* read *Parkinsonia aculeata* in the title and throughout the article.

differ in time of formation, and in thickness of wall. Those which are formed at the beginning of the season's growth, at the same time as the tracheal tubes, are relatively thin-walled and correspond to the spring wood of a typical tree. They will be described as spring substitute fibres. Those laid down in the latter part of the growth period, on the other hand, are very thick-walled, and since they are equivalent to normal summer wood will be given the somewhat awkward term of summer substitute fibres. Tracheal tubes are absent from the later wood.

In regard to the differentiation of the fibres, it is of course impossible to follow the development of individual elements. By examining numerous sections, however, a series of substitute fibres in varying stages of growth may be found, and from these a connected picture of the growth of the two types may be built up. The activity of the cambium naturally varies in the course of the season. In the non-growing period the cambium lies adjacent to the completely differentiated xylem, while during active growth a number of differentiating elements intervene between cambium and xylem and form therefore the most useful material for examination.

In observing the development of the cell wall, several microchemical tests are provisionally accepted for the identification of pectic substances, of cellulose, and of lignin. These are as follows: for pectic substances, ruthenium red; for cellulose, chlor-zinc-iodine, sulfuric acid, iodine and sulfuric acid; for lignin, phloroglucin and hydrochloric acid (2, 10).

A spring substitute fibre arises from the division of a cambial cell, and the first step in the process of differentiation is the enlargement of the cell lumen. This implies a stretching of the cell wall, but since deposition of cell wall material at this time more than keeps pace with the stretching, the cell wall actually gains in thickness. A primary wall, usually called the middle lamella, and a secondary layer are already present, the former consisting mainly of pectic substances, and the latter of pectic substances and cellulose.

Cambial activity continues meantime, and, as one daughter cell after another is cut off, a zone of differentiating xylem tissue results. At a certain point in growth a chemical change occurs in the wall of the differentiating substitute fibre. The first traces of lignification appear either intermittently or continuously along the middle lamella, and thereafter lignification spreads throughout the secondary layer. That this change in the chemical nature of the cell wall does not entail any microscopically measurable increase in cell wall thickness may be seen in figure 1 (pl. X). Here is illustrated a zone of differentiating xylem tissue, in which the cells to the left of *A–B* are unlignified, while in those to the right of *A–B* lignification has taken place. Microscopically it is impossible to measure any difference in thickness

between unlignified cell wall *6* and the adjacent lignified cell wall 7.

The differentiating spring fibre with its lignified wall is still a living cell with nucleus, chondriosomes, and small starch grains. The protoplast continues for a time to add to the thickness of the lignified wall, but finally ceases with the formation of a tertiary lamella. This appears first of all as a very thin layer of cellulose and pectic substance, in sharp contrast therefore to the lignified secondary layer. Like the secondary layer, however, it very soon becomes more or less heavily lignified. Such is the history of the development of a spring substitute fibre. The progressive increase in wall thickness is illustrated in figure 2 (pl. X), where the walls range from 1.1 microns in the cambium to 2.5 in the starch-containing differentiated substitute fibre.

In regard to the differentiation of a summer substitute fibre, the early stages of growth are similar to those of the spring fibre just described. Primary, secondary and tertiary layers appear in like succession, but at this point a change in metabolism occurs in the case of the summer fibre. The tertiary layer of the latter increases enormously in thickness, often exceeding the sum total of the previous layers. Pectic substances, cellulose and lignin are present as before in varying degrees. As the growth season draws to a close a final layer is deposited on the rapidly narrowing cell lumen. Treatment with sulphuric acid indicates that this layer is actually composed of two lamellae radially striated, as seen in figure 4 (pl. X). The wall when complete varies from 2.5 to 4.5 microns in thickness. When the wall of a summer substitute fibre is treated with certain microchemical reagents reactions of the various layers take place. These are summarized in Table I.

Layers iv and v: after the disappearance of layers ii and iii in concentrated sulphuric acid, the innermost lining of the fibre remains in the center of the cell lumen, and in it two distinct lamellae are distinguishable both with well marked radial striations (pl. X, fig. 4).

In the wall of such a fibre there are therefore five layers distinguishable by microchemical tests, in which the distribution of the components lignin, cellulose, and pectic substances may be diagrammatically represented (pl. X, fig. 5). It is seen that while lignin is present in all layers, cellulose is indicated only in layers ii to v, and pectic substances are distinguishable only in layers iii to v. In connection with the absence of pectin from layers i and ii, it has already been remarked that in the unlignified walls pectic substances are present in these layers. When lignification occurs it would therefore appear that all the pectic substances present in layers i and ii are converted *in situ* into lignin.

TABLE I

	Phloroglucin and HCl	Chlor-Zinc-Iodine	IKI and H_2SO_4	Ruthenium Red	H_2SO_4
Layer i (middle lamella)	red	orange	brown	colorless	becomes brown, does not swell, remains as network
Layer ii	pink	yellow	pale blue	colorless	becomes bluish, swells, turns buff, and dissolves
Layer iii	bright magenta	mauve	deep blue	deep red purple	becomes blue, swells strongly, dissolves
Layer iv	pale pink, thin, difficult to distinguish from iii	yellow, similar to ii, not quite so wide as iii	pale blue	light red difficult to distinguish from iii	(See text for explanation.)
Layer v	not distinguishable except with H_2SO_4				(See text for explanation.)

A notable feature in the stem and root of the palo verde is the abundance of starch present in the substitute fibres. Starch grains, as already noted, are present in the differentiating substitute fibres. They increase gradually in size until they may finally block the entire cell lumen except for small intervening spaces in which nuclei and protoplasm may be detected (pl. X, fig. 6). At the beginning of the growing season it was observed in certain cases that part of the starch reserves was being utilized, for the starch grains of the last year's xylem were considerably smaller than in the resting season.

DISCUSSION

The chemical structure of the substance lignin is still a vexed question among the chemists (7), and microchemical methods, as at present developed, can shed little if any light on this particular problem. From a microscopic study of the development of xylem fibres, certain facts emerge which may elucidate the process of lignification from the biological standpoint.

It is generally agreed that the cell wall, as it is first formed in a dividing cambial cell, consists mainly of pectic substances.

It is observed that in the differentiating tissues sugars are abundant. Accepting provisionally the tentative formula of Dore (3) for pectin, and also its colloidal nature, we may visualize a series of pectin molecules being laid down by the active protoplast. These complex molecules, hexa-rings of galactose, arabinose, and galacturonic acid, are haphazard in arrangement and are held together by van der Waal's forces. They form an extremely plastic partition of optically isotropic material. Activity of the protoplast presumably varies at different points on the cell surface, and since deposition will occur now at one point, now at another, a series of pectic flakes will result. When deposition temporarily ceases, proteins, carbohydrates, fats and lipoid materials, present in or near the surface layer will come in contact with the pectic flake, and will temporarily adhere. Meantime deposition is active in the surrounding areas, and the adjacent pectic flakes may overlap the non-pectic materials, thus enclosing them. The result is an incompletely coordinated mesh-work of pectin materials, with various other carbohydrates and proteins in the interstices.

A change is now initiated, accompanying the osmotic swelling of the cell, and the consequent stretching of the cell wall. Cellulose condensation begins to take place, and the cellulose, like the pectin, may be considered to be deposited in flakes, but in this case the flakes are made up of regularly oriented longitudinal chains as described by Sponsler (9). These flakes behave as optically anisotropic substances, and may be regarded as forming the stabilizing scaffolding in the plastic wall. At this stage of development there is a constant alternation on the part of the protoplast between pectin and cellulose formation.

Since the mesh-work of the growing wall is, as we have seen, sufficiently loose to include various organic compounds, it is not unreasonable to suppose that a specific enzyme, which we may provisionally term lignase, is present within the complex.

Meantime the activity of the cambium continues, and the first formed differentiating xylem element is removed from the main source of carbohydrate supply in the phloem by a succession of intervening cells. Across the differentiating xylem elements there thus arises a decreasing osmotic gradient.

During this period of intensive growth the transpiration current in the xylem reaches its maximum tension, and the transpiration pull will tend to withdraw water from the surrounding walls. The cell walls of the differentiating elements next to the xylem will therefore be subjected to a struggle for water, due to an osmotic pull outward and to the transpiration pull on the inner side.

It is an observed fact (6, 8), demonstrated readily by thionin and other indicators, that across the phloem, cambium and xylem there runs another gradient, that of hydrogen ion concentration. It is also known that the majority of enzymes function only

within a very narrow range of pH values. It would appear, therefore, that the functioning of the postulated enzyme lignase may be conditioned first of all by the pH value, and secondly by the water content of the cell wall, since a relative deficit of water might allow of the closer interaction of the effective substances. The process of lignification may then be regarded as a condensation reaction *in situ* taking place among the imprisoned carbohydrates, mainly pectic in nature, within the cellulose framework, and activated by a problematical enzyme lignase. The water molecules set free during the condensation process are absorbed by the protoplast itself, or are withdrawn in either the transpiration or the osmotic streams already mentioned.

If we turn to other areas in the plant where lignification is in process intermittently, for example in the zone of phloem fibre differentiation, we can see that the conditions which obtain there may be interpreted in a similar way. The phloem, due to the presence of food materials, is a region of high osmotic pressure, and next to it lies the photosynthetic cortex. The latter, during its active phases, will tend to withdraw water from every available surrounding source, and to this extent will resemble the transpiration stream of the former case in its effect. The cells on the margin of the phloem, like the cells of the differentiating region of the xylem, thus lie in a position difficult as regards water supply. Further, in the tissues at this point, there is again apparent a gradient of hydrogen-ion concentration, though in the inverse direction. Presumably the conditions in relation to enzyme activity are here similar, at least during the intermittent formation of lignified phloem fibres, to those in the zone of differentiating xylem.

That an enzyme is present during the process of lignification appears to be a reasonable proposition and should allow of direct experimental verification. Enzymes, generally speaking, catalyze either the building up or the breaking down of a particular substance. Now Alexandrov (1) in his work on hemp has shown that delignification of the xylem tissue may be consistently induced by the twisting, without breaking, of the branches of actively growing hemp. From material such as this it should be possible to isolate the enzyme concerned, if this be present.

Conclusions

1. The main bulk of the wood of the palo verde consists of substitute fibres, the walls of which vary in thickness.

2. By ordinary microchemical tests three gross layers may be distinguished in the relatively unthickened walls of the spring substitute fibres, while five are present in the very thick-walled summer fibres. These layers differ in optical and in staining properties, according to their pectic, cellulose, and lignin content.

3. The developing cell wall may be regarded as a very loose mesh-work of pectic and cellulose materials, the cellulose flakes acting as a structural scaffolding. Other non-carbohydrate substances may be enclosed within the mesh during formation.

4. Lignification, the condensation of pectic or similar carbohydrate substances, takes place *in situ* in the cell wall at a certain stage of development. The controlling factors suggested are the hydrogen ion concentration and the water content of the differentiating wall. The latter depends on the balance between transpiration pull in the xylem and osmotic forces in the differentiating phloem tissues.

5. An enzyme lignase is postulated to activate the reaction. Experimental proof of the presence of such an enzyme might be obtainable from the examination of tissues in which delignification is in progress.

6. The conditions for the lignification of the phloem fibres are seen to be essentially similar to those of the xylem elements.

7. Starch grains are present in the differentiating elements and completely block the lumen of the still living fibres at the end of the season.

University of California at Los Angeles,
August, 1935.

Explanation of the Figures. Plate X

Fig. 1. Actively growing stem: cambium *C;* unlignified differentiating xylem, *1* to *6;* partly lignified xylem elements, *7* to *10*. *A–B* is the zone of division between lignified and unlignified areas. (The uniformity in thickness of all the cell walls is seen.) (Camera lucida × 500.)

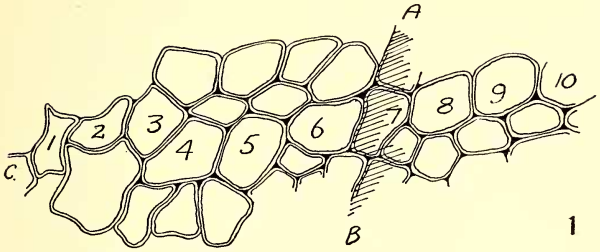
Fig. 2. Transverse section showing wall thickness in cambium *C,* and in unlignified (*1, 2*) and lignified (*3, 4, 5, 6*) differentiating spring substitute fibres. Growth less active than that illustrated in figure 1. Wall thickness in microns: 1.1, 1.1, 1.6, 1.6, 1.8, 2.5, 2.5. Starch grains *S*. Cell wall layers, *i, ii,* and *iii*. (Camera lucida × 500.)

Fig. 3. Transverse section showing cambium *C* and xylem (shaded) at the end of the growing season. In the walls of summer substitute fibres, five layers are indicated: *i, ii, iii, iv, v*. Thickness of the walls 2.5 to 4.5 microns. (Camera lucida × 500.)

Fig. 4. Transverse section of thickened fibre after treatment with sulphuric acid. Middle lamella, *m.l.,* layers *iv* and *v*. Diagrammatic.

Fig. 5. Longitudinal section of thickened fibre wall, showing the distribution of lignin, *L;* cellulose *c;* and pectic substances *p*. Diagrammatic

Fig. 6. Xylem fibre: starch grains *S*: protoplasm, *ppm;* nuclei, *n;* pits, *p*. Length from 240 to 480 microns. Diagrammatic.



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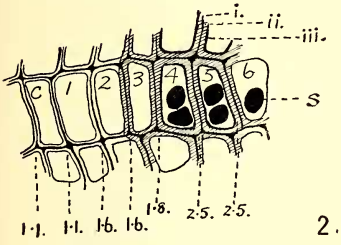

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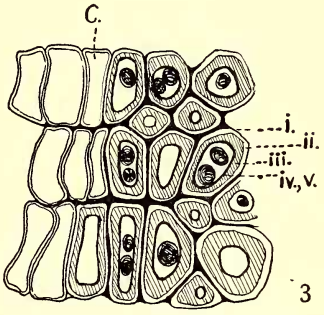

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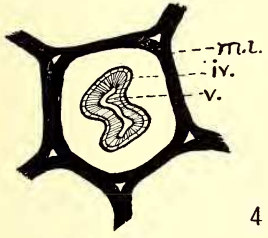

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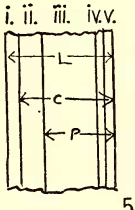

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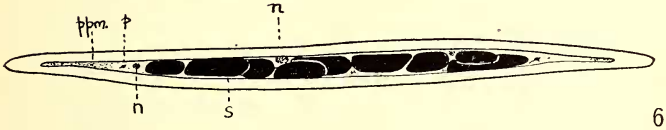

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PLATE X. PARKINSONIA ACULEATA: LIGNIFICATION OF XYLEM

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OUR VANISHING LICHEN FLORA

Albert W. C. T. Herre

Lichens are perhaps the least studied of any considerable group of plants. For convenience they may still be regarded as a group, although they are really a heterogenous assemblage that should be distributed among the fungi. Most writers of general texts on botany dismiss lichens with a few casual remarks replete with misinformation and inaccuracies. It has been said that lichens are the most difficult group of plants, but this is a gross overstatement. One has but to contemplate some of the fungi, Leguminosae (*sensu lato*), Compositae, Orchidaceae, or other large assemblages, to recognize that there are plenty of puzzles among plants other than lichens.

Yet despite the real or apparent difficulties of lichen study, and the vast distance of California, in early days, from centers of study or research, the lichen flora of the state long ago attracted the attention of European and New England botanists. No other part of our country has such a large number of endemic lichens notable for size, unusual thalline development, or other peculiarities which force them upon our attention. Then, too, a number of European species occur in California, although not in the regions east of the Sierra Nevada. Menzies, Bolander, and other keen observers supplied Tuckerman with specimens which the latter described. Later, Dr. H. E. Hasse and the writer made large collections of the lichens peculiar to California. When I left California in 1912 it was still possible, with one or two exceptions, to collect endemic lichens in their type localities.